INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DOI: https://dx.doi.org/10.33289/IJRPC.12.2.2022.12(14)

TOTAL PHENOLIC, FLAVONOID CONTENTS AND FREE RADICAL SCAVENGING ACTIVITY OF *ASTRAGALUS ARMATUS* WILLD. ETHANOLIC EXTRACT

Mounira Merghem* and Wafa Nouioua

Laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Natur and Life Sciences, Ferhat Abbas University, Sétif-1, Algeria.

ABSTRACT

Astragalus armatus Willd. (Fabaceae) is spontaneous plant found in North Algeria. In the present study the evaluation of the antioxidant activity of Astragalus armatus ethanolic extract was evaluated using the β-carotene/linoleic acid system and the DPPH scavenging method. The polyphenolic and flavonoids contents of Astragalus armatus ethanolic extract was found to be 173.15 µg GAE/mg extract and 31.07 µg QE/mg extract, respectively. The scavenger effect of ethanolic extract against DPPH radicals showed IC50 value of 0.173 ± 0.001 mg/ ml. The β-carotene bleaching assay indicated a strong inhibition percentage of the lipid peroxidation with a value of 77.456 ± 1.921%. This study revealed that the ethanolic extract had the strongest antioxidant activities, which were correlated with its high level of phenolic and flavonoids content.

Key words: *Astragalus armatus*, antioxidant activity, polyphenols, DPPH scavenging, β-carotene.

1. INTRODUCTION

Medicinal plants have been used since ancient times to relieve and cure diseases human. In fact, their therapeutic properties are due to the presence of thousands of bioactive natural compounds. There is increasing trend in correlating phytochemical constituents of plants with its pharmacological activities. Leaves, flowers, stems, roots, seeds, fruit and bark can all be constituents of herbal medicines[1]. Since the middle of the nineteenth century, different classes of bioactive compounds have been isolated and characterized. Many of these are used as the active ingredients of modern medicines, or as the lead compounds for new drugs discovery. Several plant derived medicines are rich in phenolic compounds, flavonoids, balkaloids, tannins etc., used in the treatment of various degenerative ailments[2,3].

Reactive Oxygen Species (ROS), contribute to cellular aging, mutagenesis, carcinogenesis, and coronary heart disease, likely through destabilization of membranes, DNA and protein damage, and oxidation of low-density lipoprotein (LDL)[4]. Mechanism of action of antioxidants includes the suppression of ROS formation, the inhibition of enzymes or chelating of elements involved in free-radical production. Furthermore, antioxidants scavenge reactive species, and upregulate antioxidant defences[5].

Phenolic compounds are ubiquitous in plants. Flavonoids and other plant phenolic, such as phenolic acids, stilbenes, tannins, lignans, and lignins, are important in the plant for normal growth development and defense against infection and injury. These compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity[6].

The Fabaceae are a large family of flowering plants, including shrubs and perennials or annuals herbaceous plants, currently used in traditional medicine[7]. Astragalus is the largest genus of Fabaceae including about 3000 well-known species, found in arid, semi-arid, and continental zones of western North America, Central Asia, North and South Africa[8]. In North African flora, there are ten Astragalus species that are endemic to Tunisia, Morocco and Algeria. In folk medicine, Astragalus species are used against chronic bronchitis, stomach ulcer, cough, hypertension, diabetes,

gynecological disorders and venomous bites of scorpion[9].

The biologically active sources of *Astragalus* species comprise polysaccharides, saponins, phenolics and the toxic compounds; such as nitrotoxins, imidazoline alkaloids and selenium derivatives[10]. However, researchers have mainly focused on the isolation and elucidation of cycloartane-type triterpenes[11,12]. The aim of this work was to quantify the content of phenolic compounds and to study the *in vitro* antioxidant activity of the ethanolic extract of *Astragalus armatus.*

2. MATERIALS AND METHODS

2.1. Chemicals

Folin-Ciocalteu, aluminum chloride ($AlCl_3$), gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), gallic acid and tween 40 were purchased from Sigma Chemical Co. (St. Louis, MO). Linoleic acid, β-carotene and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical Co. (Buchs, Switzerland).

2.2. Plant material

Astragalus armatus was collected from Amoucha région, Wilaya of Sétif Northeast of Algeria

2.3. Preparation of plant extract

2.3.1. Ethanolic extract

The ethanolic extract was obtained by maceration in water/ethanol mixture (20:80) for 24 h. The resultant extract was filtered through Wattman paper and the solvent was removed by rotary evaporator under reduced pressure at 45°C.

2.4. Determination of total polyphenol content

Total phenolic content was determined using Folin-Ciocalteu method, according to Li and al,[13] with slight modifications. A volume of 100 µl of the extract was mixed with 500 µl of Folin–Ciocalteau (diluted 10% in distilled water). After 4 min, 400 µl of sodium carbonate solution Na_2CO_3 (75 g/l) was added to the mixture, the reaction mixture was incubated at room temperature for 1h 30 min and the absorbance of the mixture was measured at 760 nm, Gallic acid (20-140 mg/l) was used as standard for the calibration curve. The total polyphenols content was expressed as micrograms of gallic acid equivalents (GAE) per milligram of extract. All samples were analyzed in three replications.

2.5. Determination of total flavonoids contents

The total flavonoids in plant extracts were determined using the aluminum trichloride ($AlCl_3$) method[14]. Briefly, 1 ml of 2% $AlCl_3$ in methanol was mixed with 1 ml of the extract. After incubation in dark at room temperature for 10 min, the absorbance of the reaction mixture was measured at 430 nm. Quercetin (1-40 mg/l) were used as standards for calibration curve and the total flavonoids content was expressed as micrograms quercetin equivalents (QE) per milligram of extract.

2.6. Evaluation of antioxidant activity

2.6.1. DPPH free radical-scavenging assay

The free radical scavenging activity of the extracts was measured by 2,2- diphenyl-1-picrylhydrazyl (DPPH) assay[15]. After dissolving the extracts, the solution of DPPH in methanol (0.04mg/ mL) was prepared and 1250 µL of this solution was added to 50µL of extracts solution at different concentration and kept in the dark for 30 minutes at room temperature. Then, the absorbance of this solution was measured at 517nm. All tests were performed in triplicate. Radical-scavenging activity was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

A_{blank}: Absorbance of the control.
A_{sample}: Absorbance of extract.

2.6.2. β-carotene/linoleic acid assay

In this test, the antioxidant capacity of the extracts was determined according to the method described by Kartal et al[16]. The β-carotene solution was prepared by dissolving 0.5 mg β-carotene in 1 mL of chloroform, one milliliter of this solution was pipetted to a flask covered with aluminum foil. Then 25 µL of linoleic acid and 200 mg of tween 40 were added in the foil and the chloroform was evaporated using evaporator. After evaporation, 100 mL of distilled water saturated with oxygen was added. Then 2.5 mL of this mixture were transferred to test tubes, and 350 µL of the extracts (2mg/mL methanol) were added and the absorbance was reading at 490 nm after 1h, 2h, 6h, 24h and 48h respectively. The same procedure was repeated with butylated hydroxyl toluene (BHT) as a positive control and with distilled water as a negative control. The antioxidant activity of extracts was calculated using the following equation:

$$AA\% = A_{sample} / A_{BHT} \times 100.$$

A_{sample}: Absorbance of the Extract.
A_{BHT}: Absorbance of positive control BHT.

Statistical Analyses
The results are expressed as the mean ± standard deviation. One-way analysis of variance (ANOVA) was performed to assess differences between groups.

3. RESULTS AND DISCUSSION

3.1. Total polyphenols and flavonoids contents

The results of Total polyphenols and flavonoids contents are shown in Table 1. From these results, ethanolic extract showed high phenolic and flavonoid compounds (173.15 µg GAE/ mgE and 31.07 µg QE/ mgE respectively)

Table 1: Total polyphenols and flavonoids content of *Astragalus armatus ethanolic* extract

Extract	Polyphenols	Flavonoids
	µg GAE/mg extract	µg QE/mg extract
EE	173.15	31.07

EE : ethanolic extract, GAE: gallic acid equivalent, QE: quercetin equivalent. Each value represents the mean ± SD (n = 3).

Phenolics are the most wide spread secondary metabolites in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelator. It has been reported that the antioxidant activity of phenols is mainly due to their redox properties, hydrogen donors and singlet oxygen quenches[17] and they are very important plant consistuents because of their scavenging ability, which is due to their hydroxyl groups[18].

3.2. Antioxidant activity evaluation

3.2.1. DPPH radical scavenging activity

DPPH• is one of the free radicals widely used for testing preliminary radical scavenging activity of the plant extract[19].The antioxidant properties of *Astragalus armatus* ethanolic extract was evaluated by DPPH assay, the results was expressed as IC_{50} values and shown in table 2. The ethanolic extract showed remarkable scavenging activity (IC_{50}= 0.173 ± 0.001 mg/mL)

Table 2: DPPH scavenging activity of *Astragalus armatus* extract and standards

Extracts	IC_{50}(mg/mL)
EE	0.173 ± 0.001
Gallic acid	0.001 ± 0.000#
BHT	0.043 ± 0.003#

#: µg/ml. Each value represents the mean ± SD (n = 3).

The antioxidant activity is based on the redox properties of the extracts which facilitate their activity as reducing agents; such ability is generally associated with the presence of reductants which exert antioxidant action through breaking the free radical chain by donating a hydrogen atom or preventing peroxide formation[20].

3.2.2. β-carotene/linoleic acid bleaching assay

The results of inhibitory activity of *Astragalus armatus* ethanolic extract on β-carotene bleaching are presented in table 3.The highest inhibition was provided by BHT (100 ± 3.972), followed by *Astragalus armatus* ethanolic extract (77.456 ± 1.921).

Table 3: Antioxidant activities of *Astragalus armatus* extracts at 24 hours of incubation measured by β-carotene bleaching method.

Extracts	Inhibition %
EE	77.456 ± 1.921
BHT	100 ± 3.972
H2O	30.91 ± 3.864

Each value represents the mean ± SD (n = 3).

The β-carotene bleaching method is based on the loss of the orange colour of b-carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants[21]. However β-Carotene has been reported to quench or inhibit directly radical species and lipid peroxidation in liposomes, thereby acting as an antioxidant by protecting cells and organisms from oxidative damage[22, 23]

4. CONCLUSION

The ethanolic extract of *Astragalus armatus* showed very high total phenolic content and is potent primary antioxidant as shown by their high radical scavenging capacity.

5. REFERENCES

1. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine. 2006;27:1-93.
2. Uddin G and Rauf A. Phytochemical screening and biological activity of the

aerial parts of Elaeagnus umbellate. Sci ReEssays. 2012;7:3690-3694.
3. Lakshmi GM, Bhuvaneshwari V and Amsaveni R. Antioxidant and antibacterial activity from whole plant ofEclipta alba (L.) - an in vitro model. IJBSANS. 2015;2(1).
4. Heim KE, Tagliaferro AR and Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structureactivity relationships. J Nutr Biochem. 2002;13:572- 584.
5. Montoro P, Braca A, Pizza C and De Tommasi N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chem. 2005;92:349-355.
6. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS and Heinonen M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. Journal of Agricultural and Food Chemistry. 1999;47:3954–3962.
7. Hernandez V, Morta F and Mele´ndez P. A study of medicinal species and their ethnomedicinal values in Caparo Barinas, Venezuela. Emirates Journal of Food and Agriculture. 2012;24128–132.
8. Lock JM and Simpson K. Legumes of west Asia: a check-list. Royal Botanic Gardens, Kew. 1991.
9. Bellakhdar J. La Pharmacopee Marocaine Traditionnelle. Paris (France): Ibis Press. 1997.
10. Pistelli LF. Secondary metabolites of genus Astragalus: structure and biological activity. Nat Prod Chem. 2002;27:443–545.
11. Polat E, Bedir E, Perrone A, Piacente S and Alankus-Caliskan O. Triterpenoid saponins from Astragalus wiedemannianus Fischer. Phytochemistry. 2010;71:658–662.
12. Savran T, Gulcemal D, Masullo M, Karayldrım T, Polat E, Piacente S and Alankus-Caliskan O. Cycloartane glycosides from Astragalus erinaceus. Rec Nat Prod. 2012;6:230–236.
13. Li HB, Cheng KW, Wong CC, Fan KW, Chen F and Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry. 2007; 102:771-776.
14. Bahorun T, Gressier B, Trotin F, Brunete C, Dine T, Vasseur J, Gazin JC, Pinkas M, Luycky M and Gazin M. Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparation. Drug Research. 1996;16.
15. Burits M and Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytotherapy Researc. 2000;14:323-328.
16. Kartal N, Sokmen M, Tepe B, Daferera D, Polissiou M and Sokmen A. Investigation of the antioxidant properties of Ferula orientalis L. using a suitable extraction procedure. Food chemistry. 2007;100:584–589.
17. Narayanasamy K and Ragavan B. In vitro antioxidant activity of Zanthoxylum tetraspermum W.A. stem bark. Int J Engi Sci Tech. 2012;4:155-162.
18. Hatano T, Edamatsu R and Mori A. Effect of interaction of tannins and related poyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 1989;37:2016-2021.
19. Bhuiyan MAR, Hoque MZ and Hossain SJ. Free Radical Scavenging Activities of Zizyphus mauritiana. World J Agr Sci. 2009;5:318-322.
20. Kumaran A. Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. Food Chem. 2006;97:109–114
21. Kulisic T, Radonic A, Katalinic V and Milos M. Use of methods for testing antioxidative activity of oregano essential oil. Food Chem. 2004;85:633–640.
22. Packer JE, Mahood JS, Mora-Arellano VO, Slater TF, Wilson RL and Wolfenden BS. Free radicals and singlet oxygen scavengers: Reaction of a peroxyl-radical with β-carotene, diphenyl furan and 1,4-diazobicyclo(2,2,2)-octane. Biochem Biophys Res Commun. 1981; 98:901–906.
23. Krinsky NI and Deneke SM. Interaction of oxygen and oxy-radicals with carotenoids. J. Natl. Cancer Inst. 1982; 69:205–210.